Early Journal Content on JSTOR, Free to Anyone in the World

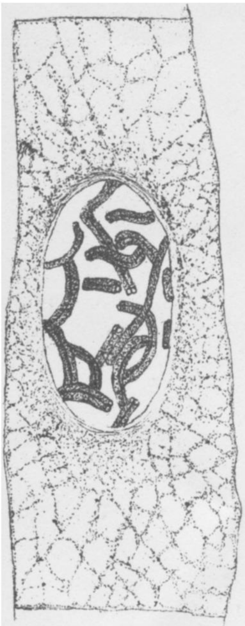
This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

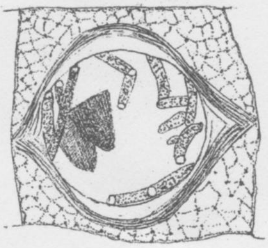
We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

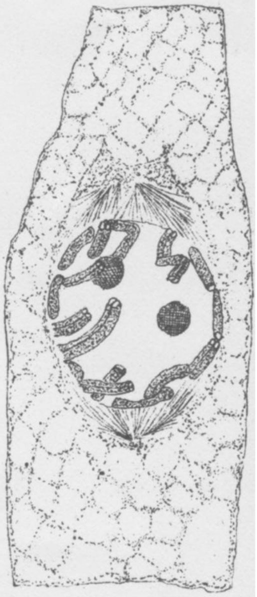
JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.



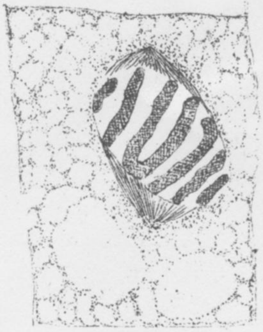
1



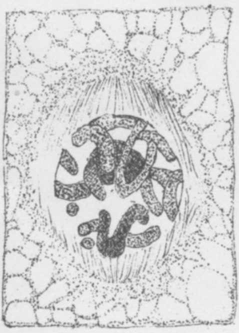
2



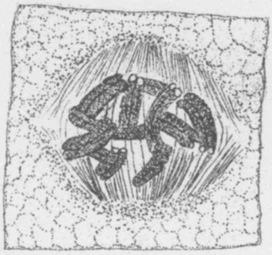
3



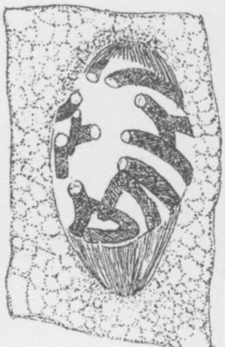
5



6

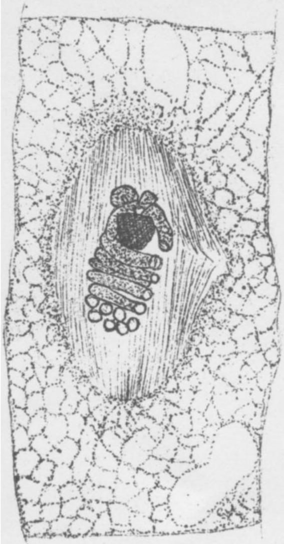


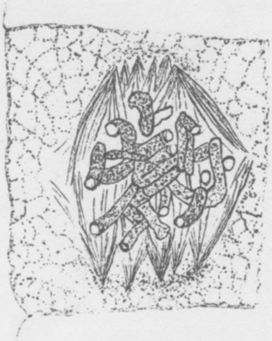
7

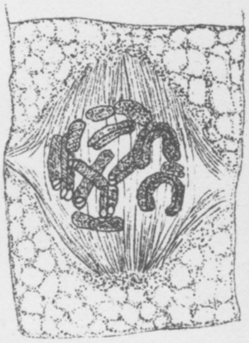


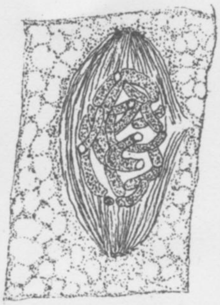
4

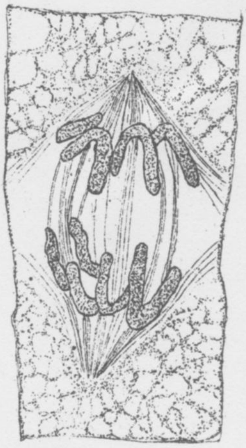
THE HELIOTYPE PRINTING CO., BOSTON.

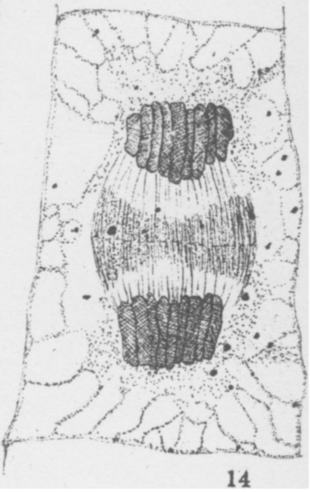

10

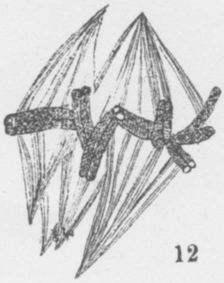

11


8


9


13


14


12

THE HELIOTYPE PRINTING CO., BOSTON.

The Development of the Karyokinetic Spindle in Vegetative Cells of Higher Plants

BY AMANDA McCOMB

(WITH PLATES 24 AND 25)

Until recent years very little was known in regard to the development of the nuclear spindle in higher plants. The commonly accepted view, that the karyokinetic spindle arose through the instrumentality of centrosomes or centrospheres was shown to be incorrect by the researches of Mottier ('97) and Osterhau ('97) upon spore-mother cells of certain higher plants. This discovery gave an impetus to the study of karyokinesis in vegetative cells, and during the last few years a number of observers have devoted their entire attention to this subject.

Nemec ('98) who has recently published an account of the origin of the spindle in vegetative cells states, that when the chromatin thread divides into chromosomes a hyaline court appears about the nucleus. In *Allium cepa* it is never of uniform width around the nucleus, but it is broader on the sides turned towards the poles of the future spindle.

This court is sharply differentiated from the surrounding cytoplasm by a definite membrane. When the chromosomes are assuming an equatorial position this membrane begins to disappear. In this hyaline space or court central spindle fibers originate, while the peripheral fibers owe their origin to the membrane.

"Zu der selben Zeit," to use his exact words, "wo der Kernfaden in die Chromosomen zerfällt, erscheint um den Kern herum ein hyaliner Hof, der jedoch zunächst an den Polen der Theilungsachse sicher zu constatiren ist (l. c., Fig. 34, Taf. III.). Der Hof ist gegen die ihn umgebende Plasmaansammlung scharf abgegrenzt. * * * Dasselbe scheint auch seine eigene Membran zu besitzen (l. c., 34, Taf. III.), die jedoch im Stadium wo sich die Chromosomen zur Aequatorialstellung umzulagern beginnen, verschwindet."

" Die Spindelanlage tritt ueberall als ein hyalines, den kern umgebendes Gebilde auf, das an den Polenkappenförmig geformt ist."

The mantle fibers, which, according to the same observer, are first noted with the appearance of the hyaline court, arise in the cytoplasm immediately surrounding the court. These fibers grow gradually from the poles and on reaching the cell walls fasten themselves there.

Soon after the appearance of Nemec's paper, Hof ('98) published an account of his researches in an article entitled " Histologische Studien an Vegetationspunkten."

He found nothing that indicated the origin of the nuclear spindle until at the time of the segmentation of the chromatin thread. When first noted the spindle anlage, according to Hof, has the appearance of caps of kinoplasmic fibers at two diametrically opposite points on the outer surface of the nucleus. These kinoplasmic fibers, which he calls the pole caps, from their reaction toward reagents, show themselves to be the beginning of the nuclear spindle.

These caps take the form of a sharply pointed cone in the inner part of which are delicate threads that are fastened to the nuclear wall and converge to one point, whereby a bipolar spindle is formed.

Not until the chromatin thread is segmented into the chromosomes does he observe any indication of the karyokinetic spindle. " Auf die Längspaltung der chromatischen Elemente des Fadens folgt auch seine Segmentirung in Chromosomen. Diese treten auseinander und platten sich noch mehr ab, wobei aber die Chromatin spaltung wie der unkenntlich wird und der Faden sich gleichmässig tingirt. Die Kernwandung ist noch immer unverändert vorhanden. Im Gegensatz hierzu fand Mottier, das bei der vegetativen Theilung der *Liliaceen* der Chromatin faden zur Zeit, wo die Kernnandung als solche verschwindet, und die Spindelfasern in die Kernhöhle eintreten noch nicht in seine Segmente getheilt sei. In sammtlichen von mir untersuchten Objecten verhielt sich das, wie, eben angeführt wurde, anders.

Bis in das geschilderte Stadium hinein, war an meinen Objecten, weder ausserhalb noch innerhalb die Andeutung einer Spindelanlage zu finden.

Hierauf erst werden gleichzeitig an zwei diametral-entgegenstehenden Punkten der Kernoberfläche, ihr kappenförmig aufsitzend, kinoplasmatische Ansammlungen kenntlich, * * * Dis Fasern convergiren gewöhnlich alle nach einem Punkt, dem Pol der Kappe; die Spindel-Anlage ist als dann worklich bipolar."

According to Shaffner ('98) in his paper on karyokinesis in root tips of *Allium cepa*, "The division begins with the separation of the centrospheres, and when these have moved apart nearly 180° the incept (anlage) of the achromatic spindle appears, forming two dome-shaped projections on opposite sides of the nucleus, at the summits of which centrospheres are situated, forming the poles around which are cytoplasmic radiations."

"These centrospheres gradually extend outwards, drawing the spindle into a sharp pointed bipolar structure." "The incept of the spindle is very sharply differentiated from the surrounding cytoplasm and the space between it and the nuclear membrane appears very clear and transparent, like the achromatin of the nucleus."

The mantle fibers he regards as streams of cytoplasm which have nothing to do directly with the formation of the spindle.

In a study of nuclear division in the root-tip of *Allium cepa*, *Vicia faba* and *Erythronium Americanum* my observations have shown that in many cases, at least, certain very early stages in the development of the spindle have been overlooked by the three observers just cited.

The material for this study was prepared according to the method used by Mottier ('97) in his studies of *Lilium*.

In the earliest stages observed by me the fibers which take part in the formation of the spindle are comparatively few, and difficult to differentiate from the remaining cytoplasm.

In some cases at least the spindle anlage takes its origin in a delicate kinoplasmic weft surrounding the nucleus, rather than within a clear space or court formed about the nucleus. Figs. 1 and 2 represent two very early stages. In Fig. 1 the kinoplasmic fibers present form a delicate weft about the nucleus. In Fig. 2 the weft is more pronounced and it is seen to retreat from the nucleus at two opposite points forming the cap-like spaces or courts described by Nemec. In Fig. 2 it is not assumed that the fibers which converge toward the cell wall indicate the poles of the future spindle, but they lie in the equatorial region. It is clear that the spindle shown in Fig. 8 arose from such an anlage as Fig. 2. In these first two figures mentioned, the chromatin thread had not segmented into chromosomes, so that it may be asserted with all certainty that the anlage of the spindle may appear in *Allium* before the segmentation into chromosomes.

The fibers of this weft, as they assume more and more a longitudinal direction, form cone-shaped masses of spindle fibers on opposite side of the nucleus. These cone-shaped caps may at first enclose a space nearly free from kinoplasmic fibers; but later fibers penetrate the cone- or cylindrical-shaped spaces and become attached to the nucleus membrane.

Very frequently the fibers forming the periphery of the conical cap are arranged so closely together as to give in optical section, the appearance of a membrane defining a hyaline space on opposite sides of the nucleus, similar to that described by Nemec.

In some cases, however, it seems evident that the spindle anlage does not consist of two distinct halves, but certain fibers extend from one polar region to the other, a condition which must necessarily follow in case the fibers form a weft in the beginning (Figs. 2, 3 and 6).

It may be reasonably questioned whether such stages as represented in Figs. 3, 4 and 5 ever passed through that of Figs. 2 or 1. There was nothing in the appearance of the nucleus which could justify the unqualified assertion that Fig. 3 or Fig. 4 was a later stage than Fig. 2, yet much may depend upon whether the division follows a period of longer rest or whether it was one of a more rapid succession of divisions. We shall return to this phase of the subject later on.

It is evident from Figs. 3, 4 and 5 that we have to do with mono-axial spindle anlages which are not necessarily strictly bipolar, although this is sometimes true. Frequently fibers of the anlage may converge into one point at one end and into more than one at the other (Figs. 3 and 4), but the developing spindle is not so pronouncedly multipolar as in reproductive cells (Mottier, '97).

It is a matter of frequent observation that the fibers on one side of the nucleus are much longer than those at the other

(Fig. 4). Whether this fact has any special significance can not be stated at present.

During the stage just mentioned, the chromatin thread is in the form of the hollow spirem. The longitudinal splitting has taken place earlier as described by Mottier. In all the stages following those represented in Fig. 1 until the separation of the chromosomes at the equatorial plate this longitudinal splitting exists, but it is not shown in all the figures, for the process of staining necessary to bring out the spindle fibers clearly, leaves the chromosomes too densely stained to enable one to distinguish the daughter segments.

The next important step in the process is the gradual disappearance of the nuclear membrane. This appears first on the sides next to the poles (Fig. 6). The kinoplasmic fibers enter the nuclear cavity. Many attach themselves to the chromosomes while others are seen to extend from pole to pole (Figs. 7, 8). In Figs. 6, 7, 8, the poles of the spindle are broadly truncate, although generally at this stage they are sharp pointed. The chromosomes are somewhat crowded in the equatorial region but they have not as yet become oriented into the equatorial plate of the mature spindle. This behavior of the chromosomes during the disappearance of the nuclear membrane and the penetration of the spindle fibers into the nuclear cavity, is similar to that which has been observed in pollen-mother cells (Mottier, '97).

Fig. 11 is instructive in that several distinct poles are still present in each polar region. The arrangement of the chromosomes in this figure shows clearly that it is of the same stage in development as Figs. 7 and 8. In the mature spindle here as in reproductive cells, three sets of spindle fibers may be distinguished, namely: those which extend from the poles to the chromosomes to which they are fastened, those running from pole to pole, and the so-called mantle fibers which diverge from the poles toward the periphery of the cell in the equatorial region (Figs. 8 and 11).

In the mature spindle the chromosomes are often quite regularly arranged in the equatorial plate forming the well-known monaster (Fig. 12). Frequently they do not appear to be oriented with such regularity. At this stage the spindle is as a rule strictly bipolar, but sometimes (Fig. 12) two or more poles still persist.

Another interesting phenomenon is presented in Figs. 5, 9, and 10. Here the chromatin spirem remains unsegmented at a much later period. In Fig. 5 the spindle anlage is a monoaxial bipolar structure, while the chromatin thread remains in the form of a continuous spirem. The same seems to be true also in Fig. 10, but here the nuclear membrane has disappeared and the spindle has reached a later stage in development. (The free ends shown in the drawing represents part of the spirem cut by the knife in sectioning.) The nucleolus is still present. In Fig. 9 the spirem is partly segmented. The chromosomes when in the equatorial plate are either straight rods or bent somewhat u or j-shaped. They are attached to the spindle fibers either at the ends or at the point of bending. Their evolution during metakinesis and the anaphase are so well known that a repetition here would be without value.

Although the spindle fibers may not in some cases be united into a common point or pole when the spindle is mature (Fig. 12), yet the several points or poles into which portions of the spindle fibers converge almost invariably unite into a single pole sometime during the anaphase.

Fig. 13 is a typical karyokinetic figure during the anaphase. The three sets of spindle fibers mentioned above are seen with great clearness. When the spindle fibers are relatively abundant it is readily seen that their arrangement is the same as in reproductive cells.

A marked contrast between the karyokinetic spindle in vegetative and reproductive cells in the higher plants and especially in the Liliaceae is the small quantity of spindle fibers in the former. This is one of the principal reasons why the process here is more difficult to follow. The spindle fibers are often comparatively few and difficult to stain sufficiently to enable the observer to trace them in detail, and at the same time to prevent over straining of the remaining cytoplasm which would invariably conceal the delicate fibers.

Fig. 14 shows the origin of the cell plate which is laid down here as in pollen-mother cells. The daughter chromosomes which are very regular in this particular case seem to be forming at once a daughter spirem by the union of their free ends.

From the foregoing it will be seen that the early development of the spindle does not quite agree with the observations of Hof ('98) on *Ephedra* in which it is claimed that no trace of the spindle appears until after the segmentation of the chromosomes. The segmentation of the chromatin spirem into the individual chromosomes represents a definite phase in the development of the karyokinetic process, yet the time when it occurs as measured by a certain stage in the formation of the spindle may not seem to be of much importance since it has been shown that the time of segmentation varies much under different conditions in embryo-sac and in pollen-mother-cells and we find this to be true also in vegetative cells.

In the first nuclear division in embryo-sac and pollen-mother-cells (Mottier, '97), the chromatin thread is segmented sometime before the nuclear membrane disappears and before a definite spindle is present; but in the two succeeding divisions in the embryo-sac and the second one in the pollen-mother-cell, which take place in a rather rapid succession, the nuclear membrane disappears and a definite spindle may be developed before the segmentation into chromosomes.

The first nuclear division in the pollen- and embryo-sac-mother-cells is preceded by a long period of growth in which the nucleus is in a resting stage characterized by the fine nuclear reticulum, whereas the second and succeeding divisions in the embryo-sac of *Lilium* follow a much shorter period of rest. In the pollen-mother-cell, as is well known, the nucleus does not enter the complete state of rest at all before the second division.

Hof has observed that in vegetative cells one nuclear division may follow another in rapid succession without the intervention of the period of complete rest, and we are convinced that the same often happens in *Allium*. In the light of this fact, therefore, it is possible that such conditions as revealed in Figs. 5, 9 and 10 represent nuclei which are dividing without having passed through the stage of complete rest. Here as in the embryo-sac the spindle has probably advanced farther before the segmentation of chromosomes. The possibility is also not excluded that in Fig. 14 we have the chromosomes uniting to form a hollow spirem to be a corresponding stage in a rapidly succeeding division.

In regard to the presence of centrosomes or centrospheres as claimed by Guignard and Schaffner ('98), I must assert with all emphasis that no such organs exist in the root-tip of *Allium*. In view of the development of the spindle through a multipolar structure as discovered by Belajeff ('94) and confirmed by other observers, and in the presence of well-fixed and well-stained preparations which show every step in the process it seems well-nigh incredible that any one should be able at the present day to read centrosomes or centrospheres into the process.

In view of the foregoing observations it is quite clear that the process of spindle formation in vegetative cells of the higher plants is not essentially different from that observed in reproductive cells of those plants. In both cases the spindle fibers, or at least the vast majority of them, are of cytoplasmic origin. They may either appear at first in the form of a weft about the nucleus or radiate from it. It matters little or nothing whether they radiate uniformly from the entire surface of the nucleus or are confined to two opposite sides. In the vegetative cells in question the spindle anlage may be monoaxial and strictly bipolar from the first, but it is often multipolar.

Whether the anlage be multipolar and polyaxial or multipolar and monoaxial seems to be of little importance. The spindle in all phases of its development is a product of the kinoplasmic fibers without the intervention or instrumentality of individualized kinetic centers such as centrospheres or centrosomes.

This work was carried out in the botanical laboratories of Indiana University under the direction of Prof. D. M. Mottier who so kindly gave me the benefit of his larger experience in similar investigations both in the making of preparations and in the interpretation of results.

Explanation of Figures 24 and 25

All figures drawn from microtome sections by the aid of a camera lucida and Bausch and Lomb $\frac{1}{12}$ oil immersion and oculars one inch and two inch.

All figures refer to *Allium cepa*.

Fig. 1. A cell in which a very delicate weft of kinoplasmic fibers appear about the nucleus. Chromatin thread is not segmented into chromosomes; the free ends indicate where the spirem was cut by the knife.

Fig. 2. The weft of kinoplasmic fibers is removed some distance from the nuclear membrane on two opposite sides, thus forming a colorless space between it and the nucleus. The fibers of the weft diverge on the right and left toward the cell wall.

FIG. 3. The spindle anlage consists of two cone-shaped caps on opposite sides of the nucleus. The fibers converge into one pole at the lower end, but form two or more at the upper end.

FIG. 4. Similar spindle anlage, but both poles broadly truncate.

FIG. 5. Spindle anlage similar to Fig. 4. The chromatin spirem which winds regularly in the nucleus is drawn as seen from the surface.

FIG. 6. The spindle anlage consists of a cylindrical complex of fibers in which the nucleus lies. The nuclear membrane has begun to disappear.

FIG. 7. The nuclear membrane has disappeared. The chromosomes are being oriented in the nuclear plate; the spindle poles are very truncate.

FIG. 8. Similar to Fig. 7. The mantle fibers are more pronounced.

FIG. 9. Chromatin more crowded in the center of the spindle-fiber complex. Segmentation of spirem has probably taken place.

FIG. 10. Chromatin spirem in all probability unsegmented; it is much contracted; a large nucleolus is still present. The chromatin in Figs. 9 and 10 seem to indicate that these divisions were not preceded by a period of complete rest.

FIG. 11. Spindle monoaxial but multipolar; chromosomes not quite arranged in the equatorial plate.

FIG. 12. Chromosomes arranged in the nuclear plate; the spindle is still multipolar in this case, but in this stage it is usually strictly bipolar. This is a mature spindle.

FIG. 13. Karyokinetic figure in an aphase.

FIG. 14. Chromosomes have arrived at the poles; the cell plate is being laid down in the connecting fibers.

LITERATURE

Guignard, L., '98. Les centres cintetiques chez les végétaux. Ann. des sci. Nat. Bot. III., **5**: 177. 1898.

Hof, A. C., 98. Histologische Studien an Vegetationspunkten. Botan. Centralb. **77**: —. 1898.

Mottier, D. M., '97. Beiträge zur Kenntniss der Kerntheilung in den Pollenmutterzellen einiger Dikotylen und Monokotylen. Jahrb. für wiss. Bot. **30**: 169–200. 1897.

——— **'97.** Ueber das Verhalten der Kerne bei der Entwickelung des Embryosacks und die Vorgänge bei der Befruchtung. Jahrb. für wiss. Bot. **30**: —. 1897.

Nemec, Bohumil, '99. Ueber die Karyokinetische Kerntheilung in den Wurzelspitze von *Allium cepa*. Jahrb. für wiss. Bot. **33**: 1899.

Osterhaut, W. J. V., '97. Ueber Entstehung der karyokinetisch Spindel bei Equisetum. Jahrb. für wiss. Bot. **30**: 160–167. 1897.

Shaffner, J. H. '98. Karyokinesis in the Root Tips of *Allium cepa*. Bot. Gaz. **26**: 225. 1898.